An acetylation rheostat for the control of muscle energy homeostasis

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Abstract

In recent years, the role of acetylation has gained ground as an essential modulator of intermediary metabolism in skeletal muscle. Imbalance in energy homeostasis or chronic cellular stress, due to diet, aging, or disease, translate into alterations in the acetylation levels of key proteins which govern bioenergetics, cellular substrate use, and/or changes in mitochondrial content and function. For example, cellular stress induced by exercise or caloric restriction can alter the coordinated activity of acetyltransferases and deacetylases to increase mitochondrial biogenesis and function in order to adapt to low energetic levels. The natural duality of these enzymes, as metabolic sensors and effector proteins, has helped biologists to understand how the body can integrate seemingly distinct signaling pathways to control mitochondrial biogenesis, insulin sensitivity, glucose transport, reactive oxygen species handling, angiogenesis, and muscle satellite cell proliferation/differentiation. Our review will summarize the recent developments related to acetylation-dependent responses following metabolic stress in skeletal muscle.

Key Words

- acetylation
- sirtuins
- muscle adaptation
- oxidative metabolism
- glucose homeostasis

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The skeletal muscle is an essential organ for the maintenance of mobility and whole body metabolic health. The skeletal muscle demonstrates plasticity in mitochondrial content and function, vascular remodeling, glucose mobilization, and myogenesis/atrophy following metabolic stressors such as diet or exercise. As such, metabolic stress can have both acute and chronic effects on skeletal muscle homeostasis. Metabolic sensing is therefore a crucial component of skeletal muscle adaptation to various stressors and is imperative for whole body health.

This review will examine the essential metabolic sensing pathways that are regulated by posttranslational

acetylation or deacetylation modifications that define the activity level and/or subcellular translocation of key proteins in muscle. Lysine-acetyltransferases (KATs; previously known as HATs) and -deacetylases (KDACs; also known as HDACs) regulate many proteins involved in intermediate metabolism (Guan & Xiong 2011, Patel *et al.* 2011), often by ‘sensing’ the redox state and/or the energetic state of the cell. These two states, although seemingly similar, describe different processes within the cell. The redox state often implies the $\text{NAD}^+:\text{NADH}$ or $\text{NADP}^+:\text{NADPH}$ ratio in a cell, which is correlated to the type of substrates being used to generate energy for the cell (Graham & Saltin 1989). The muscle cells that rely on glycolysis would typically be defined as having a lower $\text{NAD}^+:\text{NADH}$ ratio, while the myocytes that depend on

fatty acid oxidation for energy production have a higher NAD^+/NADH quotient (Guarente 2012). Not so divergent from this description, but an important distinction, the energetic state of a muscle conveys the state of available energy to perform work, in the form of ATP, and yet is also inherently integrated with the redox state of a cell. Given that the muscle is a large organ, whole body energy homeostasis relies to a large extent on muscle mitochondrial function for aerobic ATP production and for the maintenance of physiological redox conditions. This reliance on muscle, in turn, can prevent insulin resistance which can predispose to obesity, diabetes, and cardiovascular disease, all of which contribute to accelerated aging (Patti *et al.* 2003, Joseph *et al.* 2012).

The known and potential roles of sirtuin proteins in muscle energy homeostasis

The sirtuin proteins (SIRT1–SIRT7) are a major subfamily of the KDACs. They have become increasingly regarded as the regulators of metabolism (reviewed in Guarente (2012) and Houtkooper *et al.* (2012)). In particular, the SIRT1 homolog has been identified as a metabolically regulated protein capable of sensing cellular redox status that can then illicit mitochondrial and redox defense adaptations (Cantó *et al.* 2012). SIRT2, a predominantly cytosolic protein, has not been studied in muscle but has been shown to regulate autophagy in mammalian cells (Zhao *et al.* 2010) and differentiation in adipocytes (Jing *et al.* 2007). Recent evidence has also linked SIRT2 with a potential role in cardiac protection from ischemic injury (Narayan *et al.* 2012). The mitochondrially located sirtuins, SIRT3, SIRT4, and SIRT5, are known to modulate the acetylation status of the mitochondrial proteins and may therefore directly influence muscle metabolism and stress responses (reviewed in Pirinen *et al.* (2012)). SIRT6 and SIRT7 are preferentially nuclear proteins. Of these nuclear proteins, SIRT6 expression is elevated with exercise and plays a role in glycolytic regulation that will be discussed further in this review (Koltai *et al.* 2010, Xiao *et al.* 2010). In contrast, SIRT7 has a low expression level in skeletal muscle, but it may play a role in cardiac muscle function and plasticity (Ford 2006). Although not discussed in this review, there is also recent evidence that non-sirtuin deacetylases can act as metabolic sensors and may regulate metabolism and other processes in a metabolite-sensitive manner that is dependent on changes in coenzyme A (CoA) derivatives as well as NADPH (Vogelauer *et al.* 2012).

Despite the recent surge in sirtuin research, there are still many questions to be answered concerning the influence of these energy-sensing proteins on muscle metabolism and homeostasis. Elucidation of sirtuin pathways will certainly lead to a better understanding of the metabolic response of muscle to exercise or degenerative processes brought about by physiological stressors such as disease, aging, or unhealthy diets.

The SIRT1 and AMPK nexus regulates metabolic-dependent acetylation

An understanding of the redox state and energetic state of muscle is important during any physiological condition because the balance of these states determines the coordinated response of various energy sensors/effectors to regain muscle homeostasis. For instance, during both aerobic exercise training and caloric restriction (CR), skeletal muscle bioenergetics is coordinated by metabolic-sensing proteins, which independently detect changes in both the ratio of redox and energetic metabolites/substrates, more specifically the $\text{NAD}^+:\text{NADH}$ and $\text{AMP}:\text{ATP}$ ratios. These redox- and energy-sensing proteins include NAD^+ -dependent deacetylase sirtuin proteins (Imai *et al.* 2000), with our focus here being SIRT1, SIRT3, and SIRT6 (Houtkooper *et al.* 2010), and the AMP-activated protein kinase (AMPK; Hardie *et al.* 2012). These effector proteins are activated upon changes in the abundance of the corresponding metabolites NAD^+ and AMP. The level of these metabolites depend on a complex series of energetic (Xiao *et al.* 2007, Hardie *et al.* 2012) and redox pathways (Cantó & Auwerx 2012, Mouchiroud *et al.* 2013), along with a reliance on the quantity, type, and location of available energy substrate stores.

During exercise, muscle requires an abundant supply of ATP to maintain the rapid cycling of crossbridges between actin and myosin and the oscillatory changes in intracellular Ca^{2+} between the extracellular space and the sarcoplasmic reticulum. In addition, during aerobic metabolism NADH is extensively used to transfer electron loads from intermediary substrates to the electron transport chain to synthesize ATP via oxidative phosphorylation (OXPHOS). It is therefore evident that both AMPK and SIRT1, as AMP- and NAD^+ -sensors, are central for detecting energy stress situations during muscle metabolism.

It is through an interaction of SIRT1 and AMPK signaling pathways that muscle metabolism is tuned to adapt to the demands of energetic stress. Initial evidence demonstrating the similarity of these two effector proteins

was revealed in longevity studies. In particular, early Sir2p (the yeast SIRT1 homolog) studies suggested that this protein increased longevity in an NAD⁺-dependent manner (Tissenbaum & Guarente 2001, Anderson *et al.* 2003, Lin *et al.* 2004). Lifespan extension also occurred in *Caenorhabditis elegans* overexpressing the AMPK α subunit (AAK 2) of the AMPK hetero-trimeric complex (Apfeld *et al.* 2004). During periods of energy deprivation, AMPK is responsible for the phosphorylation of many proteins, including metabolic enzymes, such as acetyl-CoA carboxylase (ACC) for fatty acid oxidation, or the peroxisome proliferator-activated receptor- γ coactivator 1 α (PGC-1 α), a coactivator for the transcription of nuclear encoded mitochondrial proteins (Winder *et al.* 2000, Nemoto 2005, Jager *et al.* 2007, Rodgers *et al.* 2008). During energy deprivation there is also a rise in the NAD⁺:NADH ratio, which enables SIRT1 to deacetylate up to 13 potential acetyl-acceptor lysine sites on PGC-1 α , resulting in the activation of PGC-1 α (Nemoto 2005, Rodgers *et al.* 2005, Lerin *et al.* 2006).

The integration of AMPK and SIRT1 signaling, and thus the connection between AMPK activity and regulation of protein acetylation, is evident in pharmacological experiments where AICAR, an activator of AMPK, was shown to induce SIRT1 deacetylase activity (Cantó *et al.* 2009, Gurd *et al.* 2011). Although not yet shown in the muscle, experiments performed *in vivo* and *in vitro* demonstrated that AMPK activation can lead to the dissociation of the inhibitor deleted in breast cancer 1 (DBC1) from SIRT1, resulting in its activation (Nin *et al.* 2012). Furthermore, CR of mice and glucose restriction of primary myoblasts both culminate in the AMPK-mediated induction of nicotinamide phosphoribosyltransferase (NAMPT) transcription (Fulco *et al.* 2008, Cantó *et al.* 2009), the rate-limiting enzyme that converts NAM to NAD⁺ (Revollo *et al.* 2004, Houtkooper *et al.* 2010). Elevations in NAMPT expression increases the NAD⁺:NADH ratio (Preiss & Handler 1957, Kosaka *et al.* 1971, Bieganowski & Brenner 2004), thereby activating SIRT1 (Revollo *et al.* 2004, Hayashida *et al.* 2010), and thus providing a clear mechanistic link between the activation of AMPK and an increase in SIRT1 activity. Conversely, AMPK γ 3-KO animals have lower levels of NAD⁺, NAMPT, and deacetylated PGC-1 α following exercise (Cantó *et al.* 2010). The evidence is therefore convincing that AMPK controls the activity of SIRT1. In a reciprocal manner, some evidence has demonstrated that SIRT1 can also control the activity of AMPK. Through the use of an inducible SIRT1-KO model, AMPK-directed stimulation of muscle mitochondrial biogenesis by resveratrol was

shown to be dependent on SIRT1 (Price *et al.* 2012). In addition, human cultured myotubes that carry a point mutation in SIRT1 exhibit reduced AMPK activation (Biaison-Laubier *et al.* 2013). Furthermore, glucose-induced changes in AMPK activity appear to depend on SIRT1 abundance or activity in liver cells (Suchankova *et al.* 2009). Therefore, it appears that these two pathways can be independently activated but may possess overlapping and potentiating properties.

Positive regulation of acetyltransferases antagonizes the role of AMPK and SIRT1

Structural and functional studies have shown that many KATs exist as multisubunit enzymatic complexes that contain acetyl-CoA-binding domains and bromodomains. Notably, bromodomains are the only known protein modules that are able to recognize the specific lysine residues that are acetylated (Mujtaba *et al.* 2007). General control non-repressed protein 5 (GCN5) and P300/CBP-associated factor (PCAF) are members of the GCN5-related N-acetyltransferases (GNAT) family (Roth *et al.* 2001). Other important KAT families include P300/CBP (protein of 300 kDa and CREB-binding protein; Wang *et al.* 2008), MYST, named after its family members (MOZ, YBF2/SAS3, SAS2, and TIP60), and the steroid receptor coactivator (SRC) family that includes SRC1 (or NCOA1), SRC2 (TIF2, GRIP1, or NCOA2), and SRC3 (ACTR, RAC3, AIB1, P/CIP, or TRAM) (Chen *et al.* 1997, Onate *et al.* 1998). Of these KATs, GCN5, PCAF, P300/CBP, TIP60, SRC1, and SRC3 have been shown to immunoprecipitate with PGC-1 α (Puigserver *et al.* 1999, Lerin *et al.* 2006, Coste *et al.* 2008), yet the inhibition PGC-1 α through acetylation has only been shown for GCN5 and its close homolog PCAF (Lerin *et al.* 2006). GCN5 also acetylates and inactivates PGC-1 β , another family member of the PGC-1 transcriptional co-activators (Kelly *et al.* 2009).

Various regulatory nodes have been examined that help to describe the activation or deactivation of the inhibitory GCN5/PGC-1 α interaction. One potential node includes the subcellular translocation of GCN5 to the cytosol to attenuate its interaction with nuclear PGC-1 α , as has been shown to occur with an acute bout of exercise (Philp *et al.* 2011). Second, the deacetylase SIRT6 unexpectedly induces PGC-1 α acetylation through the deacetylation and activation of GCN5 (Dominy *et al.* 2012). This effect is attenuated with overexpression of a deacetylase-inactive mutant form of SIRT6. However, this finding appears to be in theoretical contention to the observed increase of SIRT6 protein in muscle with

exercise (Koltai *et al.* 2010) and the ability of SIRT6 to inhibit hypoxia inducible factor-1 α (HIF-1 α) leading to decreased glycolysis and activation of OXPHOS (Zhong *et al.* 2010). Furthermore, muscle cells from SIRT6-KO mice exhibit increased GLUT4 membrane translocation along with increased whole cell expression of GLUT1 (Xiao *et al.* 2010), thus supporting the role of SIRT6 in glycolysis. Third, GCN5 expression in skeletal muscle is increased by the transcriptional coactivator SRC3 (Coste *et al.* 2008). In line with this, germline SRC3-KO mice display increased mitochondrial function and improved glucose homeostasis and are protected against obesity. In addition, the expression of SRC3, like GCN5, is sensitive to fasting and high-fat diet (Coste *et al.* 2008). Fourth, CITED2 (CBP- and P300-interacting transactivator with glutamic acid- and aspartic acid-rich COOH-terminal domain 2) has been shown to disrupt the acetylation and inhibition of PGC-1 α by GCN5 in hepatocytes (Sakai *et al.* 2012). Upon insulin activation of PI3K/AKT signaling, the interaction between CITED2 and GCN5 is attenuated, allowing for PGC-1 α acetylation, while glucagon promotes CITED2 protein expression, releasing PGC-1 α from the negative control of GCN5. Although the influence of CITED2 on GCN5/PGC-1 α interaction in muscle has not been examined, CITED2 mRNA has been shown to decrease in muscle with insulin stimulation (Coletta *et al.* 2008). In addition, studies on mouse muscle myotubes demonstrated that CITED2 could block the effects of glucocorticoids on muscle cell atrophy. Lastly, as acetyltransferases require acetyl-CoA as a substrate for lysine acetylation, nuclear or cytosolic supplies of acetyl-CoA may regulate the activity of GCN5 or PCAF. During times of energy surplus, tricarboxylic acid cycle-derived citrate can be converted into acetyl-CoA by ATP citrate lyase, resulting in elevated nuclear and cytosolic acetyl-CoA, which have been shown to affect histone acetylation (Wellen *et al.* 2009). Analogous to this, increased levels of acetyl-CoA are postulated to also potentiate PGC-1 α acetylation through GCN5 or PCAF, although experimental evidence for this last hypothesis is lacking at present (reviewed in Jenning *et al.* (2010)).

Defining the exact signaling network that governs the activity of GCN5/PCAF in muscle function will require extensive future work, which should include the characterization of muscle-specific GCN5/PCAF single or double KO animals and potentially studies on model organisms, such as worms, which contain a single GCN5/PCAF homolog, *pcaf-1*.

The commanding role of the NAD⁺:NADH ratio in energy homeostasis

As a metabolic cofactor and a redox determinant, NAD⁺ is also required as an obligatory cosubstrate for sirtuin enzymes. The balance of the oxidized form (NAD⁺) vs the reduced form (NADH) dictates many of the functions of NAD⁺-dependant enzymes. In particular, the NAD⁺:NADH ratio influences the activity of redox reactions during the catabolism of lipids, proteins, carbohydrates, and alcohols. In addition, NAD⁺ has been shown to impact on glycolysis and OXPHOS and various fundamental regulatory functions, such as transcription, DNA repair, G-protein-coupled signaling, and intracellular calcium signaling (Bogan & Brenner 2008, Garten *et al.* 2009). With all of these essential biological functions relying on NAD⁺, the availability of NAD⁺ for sirtuin activity is limited (Rodgers *et al.* 2005, Sun *et al.* 2007, Bogan & Brenner 2008, Cantó *et al.* 2010, Hayashida *et al.* 2010, Koltai *et al.* 2010). NAD⁺ is derived from the amino acid tryptophan or from nicotinic acid or nicotinamide (NAM), which are biosynthesized *de novo* from vitamin B3 (Magni *et al.* 2004, Houtkooper *et al.* 2010, Schenk *et al.* 2011, Fernandez-Marcos *et al.* 2012). Interestingly, the NAD⁺ intermediate, nicotinamide mononucleotide (NMN), can restore NAD⁺ levels and improve glucose tolerance in high fat diet (HFD)-induced diabetic mice, when given via i.p. injection (Yoshino *et al.* 2011). Another NAD⁺ precursor is NAM riboside (NR; Rowen & Kornberg 1951), which, like NAM, can increase intracellular NAD⁺ levels via the NAD⁺ salvage pathway (Bieganowski & Brenner 2004). NR-supplemented diets have been shown to increase NAD⁺ levels and the activity of SIRT1 and SIRT3 in mice, culminating in enhanced skeletal muscle mitochondrial function (Cantó *et al.* 2012). Delivery of NR into mammalian cells occurs via nucleoside transporters, such as the equilibrative nucleoside transporters (ENT; Nikiforov *et al.* 2011). This was demonstrated through the use of the ENT inhibitors dipyrindamole or NBFI. It is therefore predicted that NR is delivered into muscle via ENT1, ENT2, and ENT3, which were shown to be expressed in the skeletal muscle (Lu *et al.* 2004).

As NAM can inhibit the different sirtuins (Bitterman 2002), the activity of the NAD⁺ salvage pathway becomes very important. The rate-limiting enzyme in the salvage pathway is the enzyme NAMPT. In fact, PNC1, the yeast ortholog of mammalian NAMPT, is essential for Sir2p-dependent lifespan extension during CR (Anderson *et al.* 2003). NAMPT most likely plays a key role in metabolism and potentially increases healthspan in mammals.

Evidence for the importance of NAMPT has been shown in cells deprived of glucose or serum (Yang *et al.* 2007) and in skeletal muscles of fasted mice (Fulco *et al.* 2008, Cantó *et al.* 2009). In addition, NAMPT expression is greatest in highly oxidative muscles and increases in expression with aerobic exercise training in both rats and humans (Costford *et al.* 2010, Koltai *et al.* 2010). These observations provide further evidence for the potential use of nutra- or pharmaceuticals to activate sirtuin-mediated deacetylation by enhancing the NAD⁺ salvage pathway or by introducing substrates for *de novo* biosynthesis of NAD⁺.

Protein acetylation status in muscle affects substrate metabolism

Skeletal muscle is highly reliant on substrates that drive OXPHOS activity. Lipids, which can be oxidized by mitochondria, are provided by lipolysis in adipose tissue and of intramuscular triacylglycerols. Carbohydrates, in the form of glucose, can be sequestered from the circulation in the post-prandial phase or following hepatic gluconeogenesis or glycogenolysis. Alternatively, glucose can also be generated by intramuscular glycogenolysis. Depending on exercise activity, muscle utilizes various energy stores ranging from glucose and glycogen to fats to synthesize ATP. As exercise intensity increases, there is a decrease in the proportion of energy derived from lipid oxidation and an increase in carbohydrate oxidation (reviewed in Graham & Saltin (1989), Holloszy *et al.* (1998), and Sahlin & Harris (2008)). Conversely, the proportion of energy derived from lipid oxidation rises significantly during prolonged exercise at low and moderate intensities.

Defects in human skeletal muscle mitochondria (Simoneau *et al.* 1995, Simoneau & Kelley 1997, Kelley *et al.* 2002, Ritov *et al.* 2005, Samjoo *et al.* 2013), reduced expression of OXPHOS genes (Mootha *et al.* 2003, Patti *et al.* 2003, Samjoo *et al.* 2013) and aberrant mitochondrial function and morphology (Kelley *et al.* 2002, Morino *et al.* 2005), all lead to the accumulation of intramyocellular lipids, which has been proposed as a potential mechanism leading to insulin resistance and diabetes. However, a recent study that examined the effects of endurance training in lean and obese men has found no definitive improvement in whole body insulin resistance despite reporting favorable mitochondrial adaptations and suggested that intramyocellular lipid profiles in obesity may only partially contribute to insulin resistance (Samjoo *et al.* 2013). Clearly, obesity-induced insulin resistance is a result of perturbations that go beyond mitochondrial biogenesis and intramyocellular lipid

oxidation. As alterations in protein acetylation have strong influences on signaling pathways related to glucose homeostasis and insulin sensitivity, this posttranslational modification may contribute to these discrepancies. For example, the acetylation substrate acetyl-CoA, the product of both glucose and fatty acid breakdown, has been shown to influence mammalian cell histone acetylation, thus highlighting the link between acetylation and metabolism (Wellen *et al.* 2009). Although it needs to be confirmed that acetyl-CoA levels directly influence the acetylation of non-histone proteins, the fact that the deacetylase activity of SIRT1 improves insulin sensitivity in diet-induced obese mice, by activating PGC-1 α amongst others, also suggests a link between protein acetylation levels and metabolism (Lagouge *et al.* 2006, Sun *et al.* 2007, Cantó *et al.* 2010). Furthermore, increased expression of SIRT1, or treatments with SIRT1 agonists, was shown to be similarly beneficial (Baur *et al.* 2006, Smith *et al.* 2009). Conforming to the involvement of SIRT1 in glucose homeostasis, cultured myotubes obtained from patients with *SIRT1-L107P* mutations exhibit both reduced glucose uptake and insulin sensitivity (Biaison-Laubier *et al.* 2013). The SIRT1-mediated effect on insulin resistance occurs, in part, through the deacetylase-dependent transcriptional repression of PTP1B at the chromatin level (Sun *et al.* 2007). As a key insulin receptor phosphatase, PTP1B can inhibit the insulin signaling transduction cascade. In addition, SIRT1 positively mediates CR-induced insulin sensitivity in muscle by deacetylating STAT3, thereby enhancing PI3K signaling and glucose uptake (Schenk *et al.* 2011). Therefore SIRT1 may not only increase β -oxidation of lipids through the activation of PGC-1 α , but may also increase insulin sensitivity in a PTP1B- and STAT3-dependent manner to improve substrate handling during times of energy stress. SIRT1 may therefore be a potential target for the prevention or treatment of diabetes in humans through its insulin sensitization effects.

Contrary to the effects of SIRT1 deacetylase activity in the nucleus or cytosol, changes in mitochondrial protein acetylation, brought about by either liver- or muscle-specific SIRT3-KO animals, had no effect on metabolic homeostasis (Fernandez-Marcos *et al.* 2012). Despite this finding, germline SIRT3-KO mice exhibited both reduced hepatic and muscle fatty acid oxidation, along with reductions in hepatic ATP production, resulting in hypoglycemia, cold intolerance, and impaired insulin sensitivity (Hirschey *et al.* 2010, Jing *et al.* 2011). SIRT3 may also counterbalance mitochondrial acetylation induced by the GCN5-like 1 (GCN5L1) protein, as was shown to be the case in liver cells (Scott *et al.* 2012).

However, given the discrepant phenotypes observed between the liver- and muscle-specific SIRT3-KO mice and the germline SIRT3-KO mice, the impact of mitochondrial protein acetylation will require further attention (Hirschey *et al.* 2010, Fernandez-Marcos *et al.* 2012). Changes in mitochondrial protein acetylation were also demonstrated in the muscle-specific KO model of the mitochondrially located carnitine acetyltransferase (CRAT; Muoio *et al.* 2012). These KO mice exhibit disrupted whole body glucose homeostasis due to the altered nutrient control of pyruvate dehydrogenase activity, without displaying changes in mitochondrial function or content. Although not a global acetyltransferase, CRAT specifically converts acetyl-CoA, within the mitochondrial matrix, into a membrane permeant acetyl-carnitine ester. Under nutrient excess, CRAT promotes the mitochondrial efflux of excess acetyl moieties that otherwise inhibit various metabolic regulatory enzymes, such as pyruvate dehydrogenase. Ultimately, the CRAT-KO mouse results in the inhibition of pyruvate dehydrogenase, which is hypothesized to slow glycolytic flux and creates a negative feedback on glucose uptake.

Evidently, mitochondrial function in muscle is important for lipid oxidation and potentially insulin sensitivity. However, evidence from the studies presented here suggests that the development of mitochondrial dysfunction only mediates part of the insulin resistance seen in muscle, with the dysregulation of insulin signaling and glucose transport via posttranslational acetylation events also being the contributing factors for the development of insulin insensitivity.

Changes in the protein acetylation status during muscle normoxia and hypoxia

Skeletal muscle is a highly metabolic tissue and is therefore highly sensitive to oxygenation. As a well-known stimulus for increasing capillarization, exercise contributes to an improved aerobic capacity that is reliant on an angiogenic response system for the induction of capillary growth. An essential mediator of this pathway, vascular endothelial growth factor (VEGF), increases in response to acute exercise (Breen *et al.* 1996, Amaral *et al.* 2001). The VEGF promoter possesses a hypoxia response element for the transcription factor HIF-1 α . However, HIF-1 α may not be the only signaling pathway to activate VEGF. Important signals for the activation of myocyte VEGF may include low partial pressure of oxygen, inflammatory cytokines, high oxidative stress, and energy balance-related AMPK activity (reviewed in Breen *et al.* (2008)). Nonetheless,

HIF-1 α is an oxygen-sensitive protein that can facilitate ATP generation in mitochondrial respiration by inducing an angiogenic response that elevates VEGF expression to match the new demand of oxygen as a substrate for oxidative respiration (O'Hagan *et al.* 2009). HIF-1 α induction of VEGF-A expression occurs with ischemic conditions in the heart and in skeletal muscles during exercise (Gustafsson *et al.* 1999, Ameln *et al.* 2005). With 4 days of chronic electrical stimulation of muscle, VEGF-A reaches its highest levels, while the capillary-to-muscle fiber ratio continually increases from 7- to 28-days of muscle stimulation (Dawson & Hudlicka 1989, Hudlicka & Price 1990, Hang *et al.* 1995). Interestingly, following PGC-1 α overexpression in primary human skeletal muscle cells and the resulting induction of mitochondrial biogenesis, there is an increase in oxygen consumption, leading to intracellular hypoxia and the stabilization of HIF-1 α (O'Hagan *et al.* 2009, Geng *et al.* 2010). However, PGC-1 α can also directly regulate VEGF and angiogenesis in a HIF-1 α -independent manner (Arany *et al.* 2008).

A sirtuin/HIF-1 α balancing act: does SIRT1/SIRT6 mediate the metabolic switch from glycolysis to OXPHOS in muscle?

Although not examined in muscle, recent findings demonstrate that SIRT1 negatively regulates HIF-1 α via deacetylation, while PCAF acetylates and activates the oxygen-sensitive protein (Lim *et al.* 2010). In addition, in mice and cells exposed to hypoxic conditions glycolysis is activated and the tricarboxylic acid cycle is inhibited, leading to decreases in NAD⁺ levels, reduced SIRT1 activity, and activation of HIF-1 α . During normoxic conditions, NAD⁺ concentrations are higher resulting in SIRT1-mediated deacetylation and inactivation of HIF-1 α (Lim *et al.* 2010). From this we hypothesize that HIF-1 α may be inactivated by SIRT1 in muscle with exercise, as exercise has been shown to increase the NAD⁺:NADH ratio and SIRT1 activity (Graham & Saltin 1989, Cantó *et al.* 2009, 2010, Costford *et al.* 2010). As discussed in the previous section, exercise may result in a temporary hypoxic environment, much like what occurs with overexpression of PGC-1 α , which would activate angiogenesis to supply the new demand for oxygen fueled by the increased mitochondrial content. Thus the induction of mitochondrial biogenesis following exercise could lead to intracellular hypoxia, leading to a reduction in OXPHOS activity, NAD⁺ availability, and the deactivation of SIRT1 and activation of HIF-1 α by PCAF (see Fig. 1).

Contraction / Relaxation **Recovery** **Figure 1**

The temporally regulated mitochondrial and angiogenic adaptations following exercise: during exercise, the skeletal muscle experiences an increase in oxidative phosphorylation and an elevation in both the NAD⁺:NADH and AMP:ATP ratio. Consequently, SIRT1 and AMPK are activated and initiate a metabolic adaptation by increasing the activity of PGC-1 α , resulting in the induction of mitochondrial biogenesis. However, these increases in mitochondria content and function have been shown to

increase oxygen consumption leading to a temporary state of intracellular hypoxia and HIF-1 α activation. With hypoxia comes a reduction in OXPHOS activity and NAD⁺ availability, which has been shown to inactivate SIRT1 and SIRT6, negative regulators of HIF-1 α . Therefore, this temporal inhibition of SIRT1 and SIRT6, along with elevated HIF-1 α activity, activates angiogenesis as an adaptation for the increased demand for oxygen following mitochondrial biogenesis.

HIF-1 α is also involved in cellular pathways related to glucose metabolism, such as glucose uptake and enzymes that regulate carbohydrate catabolism. Specifically, HIF-1 α activates transcription of the glucose transporter 1, hexokinase 1 and 2, lactate dehydrogenase, pyruvate kinase, and other enzymes involved in glucose metabolism (reviewed in Semenza (2001)). The balance between SIRT1 and HIF-1 α regulation of the metabolic switch, going from oxidative to glycolytic metabolism, is corroborated by data showing reduced SIRT1 activity in muscles of aged rats and mice due to low NAD⁺ levels, leading to an increased dependence on glycolysis (Mouchiroud *et al.* 2013). The reduction in SIRT1 activity releases the inhibition of HIF-1 α , which supports the increasing reliance of aged muscle on glycolysis for energy production (Koltai *et al.* 2010). Aged animals exhibit increases in lactate and reduced glycolytic intermediates suggesting elevated anaerobic glycolysis (Houtkooper *et al.* 2011). However, HIF-1 α action is not just controlled by SIRT1, SIRT6 also inhibits HIF-1 α and glucose uptake in muscle. Deficiency of the SIRT6 protein in skeletal muscle leads to severe hypoglycemia due to heightened HIF-1 α activity and glucose uptake along with reduced mitochondrial respiration (Xiao *et al.* 2010). Under basal conditions, SIRT6 inhibits HIF-1 α leading to decreased glycolysis and activation of OXPHOS (Zhong *et al.* 2010). Interestingly, SIRT6 expression increases with age in

unison with HIF-1 α and both proteins are reduced in these animals following exercise (Koltai *et al.* 2010). This may indicate that SIRT6 acts as a compensation mechanism during old age to limit HIF-1 α overactivity.

We propose that SIRT1 and SIRT6 coordinate their functions to regulate HIF-1 α , glucose uptake, and glycolysis in muscle. This concept has been demonstrated in macrophage precursors during an acute inflammatory response where the NAD⁺-dependent activation of SIRT1 and SIRT6 were responsible for the activation of PGC-1 α and the silencing of HIF-1 α transcription respectively (Liu *et al.* 2012). As a result, the coordination between SIRT1 and SIRT6 induces a metabolic switch from glucose metabolism to fatty acid oxidation. Future research examining the coordination of SIRT1 and SIRT6, along with the acetyltransferases GCN5 or PCAF, will help to define the potential PGC-1 α - and HIF-1 α -directed metabolic switch upon changes in oxygen demands, exercise-induced mitochondrial biogenesis, and other adaptations to metabolic stress in skeletal muscle.

Protein acetylation modulates muscle satellite cell fate

In contrast to what occurs within a differentiated muscle cell, SIRT1 acts as a regulator of peripheral satellite cell

proliferation in hypoxic conditions (Rathbone *et al.* 2009). Muscle satellite cells exposed to a hypoxic environment induce a SIRT1-mediated increase in cell cycle progression through the overall repression of cyclin-dependent kinase inhibitors, key regulators of G₁–S phase transitions. In addition, increase in the activity and expression levels of the basic helix–loop–helix (bHLH) factor MYOD marks the beginning of satellite cell activation and differentiation and is required for normal muscle regeneration (Megeney *et al.* 1996, Kanisicak *et al.* 2009). MYOD activity is controlled by multiple acetylation sites. Mice that express a mutant form of MYOD that replaces two specific lysine acetylation sites with arginine exhibit a delayed capacity for muscle regeneration *in vivo* (Duquet *et al.* 2006). In fact, the yield of myoblasts in MYOD mutant muscle was twofold higher than that found in WT animals, yet when placed under differentiation conditions, *in vitro*, there was a perturbed expression of early and late differentiation markers. MYOD can be deacetylated in the cell by a SIRT1-dependent mechanism. SIRT1 associates to PCAF/GCN5 and deacetylates PCAF and MYOD to inhibit differentiation (Fulco *et al.* 2003). From this, we can hypothesize that in satellite cells, SIRT1 acts to protect hypoxic muscle tissue by preparing the muscle for regeneration by activating the proliferation of muscle satellite cells. Concurrently, SIRT1 inhibits the differentiation of these satellite cells until the muscle is once again in a normoxic environment. Conversely, the acetylation of MYOD and activation of differentiation occur through CBP/300 and PCAF acetylase activity (Sartorelli *et al.* 1999, Polesskaya *et al.* 2000, Polesskaya & Harel-Bellan 2001). More recently, the ability of PCAF to activate MYOD was confirmed using a PCAF-specific inhibitor, embelin, which blocked differentiation of myoblasts into myotubes (Modak *et al.* 2013). Furthermore, the nuclear corepressor SMRT (silencing mediator of retinoid acid and thyroid hormone receptor) has been shown to bind and stimulate the deacetylase activity of HDAC3 toward MEF2 and PCAF causing repression of myogenesis (Grégoire *et al.* 2007). Likewise, the SMRT homolog NCOR1 (nuclear corepressor 1) recruits HDAC1 to the bHLH region of MYOD to repress gene transcription (Bailey *et al.* 1999). Importantly, muscle-specific ablation of NCOR1 in mice demonstrated enhanced exercise endurance due to an increase in muscle mass and mitochondrial biogenesis (Yamamoto *et al.* 2011). With the loss of NCOR1 *in vivo*, there was an increase in the acetylation levels and activity of MEF2, a transcription factor that potentiates the muscle-inducing activity of myogenic bHLH proteins (Yamamoto *et al.* 2011). In opposition to SIRT1-, NCOR1-, and SMRT-regulation of

MYOD, the coactivator PC4 has been shown to increase the expression of MYOD, along with the ability of this protein to stimulate muscle regeneration and myoblast differentiation (Micheli *et al.* 2011). PC4 directs the formation of a trimolecular complex of PC4, NF- κ B, and HDAC3, which deacetylates NF- κ B, an inhibitor of MYOD expression, and sequesters it to the cytosol.

These studies demonstrate that the chronological progression of deacetylase or acetyltransferase activity is important for satellite cell proliferation and differentiation. These studies also highlight the importance of the balance between these opposing enzymes when considering the development of new pharmacological approaches to activate muscle regeneration. Future studies should better define the progression and interconnections between each of the regulatory nodes of differentiation, specifically through SIRT1, PCAF/GCN5, NCOR1, and SMRT regulation of myogenic transcription factors such as MYOD and MEF2.

Reversible acetylation for the control of atrophy and autophagy

The ubiquitin–proteasome and autophagy–lysosome systems are the major proteolytic pathways that contribute to muscle wasting. The ubiquitin–proteasome system may account for up to 80% of the proteolysis during muscle wasting and is activated by the muscle-specific ubiquitin ligases ATROGIN1 and MURF1. Regulation of muscle wasting through the acetylation of NF- κ B, C/EBP β , and FOXO transcription factors was previously reported (Gutierrez *et al.* 2002, Chen & Greene 2003, Perrot & Rechler 2005). The acetylation of multiple sites on NF- κ B by P300/CBP alters the capacity of NF- κ B to bind DNA, induce its transcriptional activity, and interact with I κ B inhibitors. The transcription factor C/EBP β was shown to play a role in muscle wasting through studies on dexamethasone treatment. This synthetic glucocorticoid increases P300 binding and acetylation of C/EBP β (Yang *et al.* 2005, Chamberlain *et al.* 2012). When active, C/EBP β binds to a C/EBP β -responsive *cis*-element in the *atrogin1*/MAFbx gene promoter (Zhang *et al.* 2011). In addition, the activation of FOXO3A in skeletal muscle causes the transcription of both the *atrogin1* and *murf1* genes, followed by the induction of the atrophy program (Sandri *et al.* 2004, Sandri 2010, Senf *et al.* 2010). SIRT1 and P300 have been shown to regulate the balance between inhibition and activation of FOXO3A via deacetylation and acetylation respectively (Brunet *et al.* 2004, van der Horst *et al.* 2004, Motta *et al.* 2004). Interestingly, during glucose restriction

of mouse C2C12 myotubes, FOXO3A was also shown to form a complex on mtDNA with SIRT3 and mitochondrial RNA polymerase, thus increasing mitochondrial protein expression and respiration in response to the CR-induced atrophic signal (Peserico *et al.* 2013).

Acetylation can also regulate the process of autophagy, as was demonstrated in the dual muscle-specific KO mouse of HDAC1 and HDAC2 (Moresi *et al.* 2012). These mice exhibit a progressive myopathy that is preceded by impaired autophagic flux, yet the mechanistic link still remains elusive. Furthermore, deacetylation by SIRT1 is important for activating the autophagy-related genes *ATG5*, *ATG7*, and *ATG8* in an NAD⁺-dependent manner in mammalian cells (Lee *et al.* 2008). SIRT2 also appears to be involved in autophagy through the activation of FOXO1. In adipocytes, SIRT2 can bind and deacetylate FOXO1, a regulator of both muscle wasting and autophagy in mammalian cells (Jing *et al.* 2007, Zhao *et al.* 2010). During oxidative stress or serum starvation, FOXO1 is released by SIRT2, allowing for the acetylation of FOXO1 and the induction of an autophagic response (Zhao *et al.* 2010). Therefore, as FOXO proteins regulate key pathways during sarcopenia and muscle disuse (Sandri *et al.* 2004, Senf *et al.* 2011), SIRT2 may prove to be an interesting new avenue to manipulate autophagy and atrophy signaling in skeletal muscle. In addition, P300, which is responsible for acetylating FOXO1 *in vivo* and *in vitro*, also acts as a coactivator to potentiate FOXO1 transactivation (Perrot & Rechler 2005).

Here we emphasize the role of protein acetylation in atrophy and muscle wasting, along with the role of autophagy as a selective process for clearing dysfunctional proteins and organelles (reviewed in Menzies & Hood (2012) and Sandri (2013)). The importance of examining the role of reversible acetylation in these processes is evident from a clinical standpoint and for the basic understanding of protein and organelle turnover that exists in muscle tissue.

Conclusions

In muscle, the balance between catabolism and anabolism, substrate utilization, and oxygenation are tightly interwoven. From this discussion it should be apparent that acetylation tightly regulates these processes in a concerted manner. As a result, the muscle cell can synchronize energy status and blood flow to changes in local muscle metabolism without compromising each of these processes or the host. With the current rise in the elderly population, there is an urgent need to understand the basic mechanisms of muscle homeostasis and to delineate each

of these pathways in order to rationally tackle medical problems such as age-related loss of muscle function or glucose intolerance. In addition, much like what occurs with aging, pathologies such as muscular dystrophies, amyotrophic lateral sclerosis, diabetes, and cancer have also been defined by reductions in muscle mass, mitochondrial content and function, and the associated reduced capacity for adaptations to metabolic stress.

A better understanding of the involvement of reversible acetylation in mechanisms that underlie the pathogenesis of metabolic dysregulation has been the goal of many studies and is an important step for the development of new therapeutic approaches. Reversible acetylation orchestrates key regulatory mechanisms for both muscle and whole body metabolic homeostasis. It is apparent from the data presented here that in many cases acetylation or deacetylation cannot be simply categorized as activators or inhibitors of transcription, as once thought. These are complex systems that are context- and protein-specific. Therefore, a better understanding of these systems may offer promising approaches for pharmacological or lifestyle interventions (i.e. exercise) to modulate the activity of this integrated system, leading to the maintenance of mitochondrial content and function, energy and oxygen supply, and ultimately skeletal muscle health.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review reported.

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